T-cell dysfunction and hyperimmunoglobulinemia E in paracoccidioidomycosis

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Abstract

Various aspects of T and B cell mediated immunity were investigated in 20 well documented cases of active (10) or inactive (10) paracoccidioidomycosis (Pcm), as well as in 8 healthy individuals living in the endemic area of the disease. The results confirm previous reports that active Pcm produces diverse grades of depression of T cell mediated immunity. Such T cell dysfunction is not associated with a reduction in the number of peripheral E rosette-forming cells, and the immunodepression is reversed by chemotherapy. Sera from Pcm (active or inactive) patients have significantly increased levels of total IgE, but the actual proportion of IgE antibodies against *P. brasiliensis* was very low (0.4–0.6%). The highest levels of total IgE were found in active patients with disease-related immune depression, suggesting that T cell dysfunction might contribute to the excessive IgE production.

Introduction

Paracoccidioidomycosis (Pcm) is a progressive disease that is generally chronic and characterized fundamentally by lesions in the pulmonary parenchyma, mucosal surfaces, the skin, the reticuloendothelial system and the adrenals (Restrepo *et al.*, 1976). The causal agent is *Paracoccidioides brasiliensis,* an imperfect dimorphic fungus that adopts a yeast form at 37 °C and a mycelial form at 25 °C. The disease has only been detected in Latin America, covering a territory that extends from 23°N (Mexico) to 34°S (Argentina) (12, 27). Within the countries of the endemic area the mycosis is concentrated in regions with particular climatic conditions. The temperature generally varies between 12 and 28 °C, the average annual rainfall is from 800 to 2 000 mm, and the habitat is generally moderately to very humid forest (27).

Man is the only natural host known for *P. brasiliensis* and it is believed that the fungus exists in the environment in its mycelial form, producing spores of less than 5 μm diameter which are probably the infective particles (26). The mycosis, which is not contagious, is infrequent in children and young adults, 60% of the cases that present being persons older than 30 years. In children the prevalence is similar in both sexes, but in adults there is a marked predominance of male patients (12). From the point of view of occupation, the disease is most frequent in persons associated with agriculture. European and Asiatic immigrants who settle in the endemic area often develop the severest form of the disease (24).

In terms of its pathogenesis, it is believed that the fungus reaches the pulmonary parenchyma from its natural habitat via inspired air (15). The initial pulmonary lesion is asymptomatic in most cases, and may then either regress spontaneously, become latent, or disseminate contiguously and/or via the lymphatics (33). Various authors group the spontaneously regressing and latent forms as Pcm-'infection' and distinguish these from the progressive forms, that are termed Pcm-'disease' (11). Within

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the progressive disease group, two principal clinical types can be identified: juvenile and adult. In the first, the primary infection (pulmonary?) disseminates rapidly giving rise to acute or subacute clinical symptoms, in which the lymphoid system is predominantly involved. In adults, a chronic disease is generally developed in which the lesions may be restricted to the pulmonary parenchyma, or invasion of the teguments, the lymphoid system, the adrenals and any other organ or system can occur (24, 26).

If adequate treatment (sulphonamides, amphotericin B, ketoconazole) is not initiated, progressive Pcm can lead to the death of the patient. After treatment, fibrous lesions appear in many cases, which can cause serious functional alterations in the organs and systems affected (14).

From the immunological point of view, a range of responsiveness similar to that described in leprosy and leishmaniasis has been observed (23). In the localized or regressive forms of the mycosis delayed cutaneous sensitivity exists, and only rarely are anti-*P. brasiliensis* antibodies detected (11). In contrast, the generalized forms present high serum antibody concentrations and various degrees of depression of T-cell activity (19, 21, 23, 26). The cause of this partial immunodeficiency has been little studied; however, the recovery of T-cell activity after successful treatment (28) suggests that the abnormality is acquired, and reversible.

This report describes the results of studies of the T and B-cell responses in patients with different forms of active Pcm, in individuals with inactive disease due to treatment, and in normal individuals living in the endemic area. Our findings confirm previous reports of the existence of degrees of depression of T-cell responses, and demonstrate an association with elevated serum concentrations of IgE.

Materials and methods

We studied 28 individuals who were classified in 3 groups. Group 1 was composed of 10 patients with mycologically active Pcm, including (a) those that had not received treatment, and (b) those that were suffering relapse after abandoning their treatments. All but one of these were male, and their mean age was 40.7 years (range 16 to 56). The evolution of the disease varied from less than one to four years. According to the criteria of Yarzábal *et al.* (33), the group included three multifocal pulmonary (MFP), three multifocal disseminated (MFD) and 4 unifocal disseminated (UFD) patients. Group 2 contained 10 patients with Pcm who had been treated for at least 6 months with sulphonamides and/or amphotericin B, and whose mycologic examinations were negative at the moment of the study. The average age of the 10 males who formed this group was 47 years (range 10 to 71); the evolution of the disease ranged from 1 to 18 years, and treatment from less than 1 to 18 years. The clinical classification before treatment was comparable to group 1. Group 3 was formed by 8 healthy individuals from the endemic area, and included 5 males and 3 females, with a mean age of 39.0 (range 26–57). The geographic and racial origin, and socioeconomic level of the healthy controls were identical to that of the patients. In group 1 the diagnosis was always based upon the observation of *P. brasiliensis* in the lesions and/or its isolation by cultures and similar observations had been made in group 2 prior to treatment.

T-cell mediated immune responses

Sensitization to 1,4 di-nitro-1-chlorobenzene (DNCB)

The capacity to sensitize the patients to this hapten was studied using the technique of Waldorf *et al.* (31) as modified by Rea *et al.* (25).

Control individuals were, however, not tested, as it was considered unjustifiable to sensitize healthy individuals to DNCB. The reaction was considered positive when vesicles, induration and/or erythema, occurred at the site of application of the hapten, and that were not observed at the initial application.

Intradermic tests

(a) Antigens

Soluble antigenic extracts of *Candida albicans* B, *Mycobacterium tuberculosis* and *P. brasiliensis* were used.

To obtain the antigen of *C. albicans* B, the strain CMM* 1002 isolated and characterized in 1975 in

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the Centre d'Immunologie et de Biologie Parasitaire, Lille, France was grown in Sabouraud's broth. The antigen consisted of filtered broth, dialysed and lyophilized, from a culture that was incubated for 3 days at 37 °C with continual agitation (120 cycles per min.). The white powder resulting was redissolved in a phosphate buffered saline solution (PBS) at pH 7.2, containing 0.2% phenol. The final concentration was 100 μg/ ml. Purified protein derivative (PPD) was used as the *M. tuberculosis* antigen, and was prepared by the Instituto Nacional de Tuberculosis, Caracas, Venezuela. This was standardized to contain 1 TU of antigen per 0,1 ml.

The *P. brasiliensis* extract was kindly provided by Dr. A. Restrepo (Medellín, Colombia) and prepared by a procedure previously described (30).

(b) Procedure and interpretation

Each person included in the study was injected intradermally with 0.1 ml of each antigen solution, or PBS-phenol (as a control). The tests were read 48 hr later, measuring the perpendicular diameters of the resulting induration. Reactions with mean diameters of >10 mm for PPD or >5 mm for *C. albicans* and *P. brasiliensis* antigens were considered positive.

Enumeration of T lymphocytes

To determine the number of circulating T lymphocytes, 10 ml of venous blood was collected into 50 ml heparinized flasks and mixed with 10 ml Hanks balanced salt solution (Grand Islands Biological Co., New York, USA) containing 5 U/ ml heparin. The lymphocytes were separated on a gradient of Ficoll-Hypaque, composed of 24 volumes of 34% Ficoll (Sigma Chemical Co., Missouri, USA) and 10 volumes of Hypaque (Winthrop Products Inc., New York, N.Y., USA) as described by Boyum (2). The T-cells were counted by the rosette technique of Bach (1).

Lymphocyte transformation test

(a) Antigens

Particulate antigens were prepared from the yeast forms of the CMM 4449 strain of *P. brasiliensis,* that had been reactivated by passage in mice. The culture was obtained after 5 days in brain-heart broth (Beckton Dickinson, Co., Maryland, USA). After separation from the culture medium the cells were washed three times in PBS (0.15 M, pH 7.2), by centrifugation at 500 g. Isolated cells were then obtained using the technique of Goihman Yahr *et al.* (9). These were further washed and resuspended at 4×10^8 cells/ ml in PBS. Viability and purity was controlled by inoculation onto brain heart infusion agar and blood agar. A portion of these were stored, at 0 °C (viable particulate antigen) and the remainder autoclaved for 5 min, at a pressure of 15 pounds, 121 °C (non-viable particulate antigen).

The soluble antigen was the same as that used for intradermal tests.

(b) Procedure and interpretation

Mononuclear cells were separated from heparinized blood by sedimentation at 37 °C for 60 min and collection of the leukocyte-rich supernatant. This was diluted in an equal volume of McCoy's medium containing 100 U penicillin and 5 U heparin per ml, and placed in horizontal glass bottles. After 1 hour incubation at 37 °C the non-adherent cells were counted and adjusted to a concentration of 10^6/ ml in McCoy's medium. Phytohaemaglutinin A (PHA; Difco, Michigan, USA) at 20 μg/ ml, *C. albicans* B soluble antigen (100 μg/ ml) and particulate and soluble antigens of *P. brasiliensis* were used in the cultures.

In preliminary tests serial ten-fold concentrations from 10^3 to 10^6, then 2 and 4×10^7/ ml of particulate antigens were used. The soluble *P. brasiliensis* antigen was tested at 0.3 to 50 μg/ ml. The viable particulate antigen was not further used due to its adverse effects on 3H-thymidine incorporation by lymphocytes. The non-viable particulate antigen generated stimulation indices much higher than soluble *P. brasiliensis* antigen, thus allowing a better discrimination between individuals and groups.

The antigen selected for study was the non-viable particles at a concentration of 10^6 cells/ ml. The cultures were performed in triplicate and incubated with the various antigens for 6 days, and PHA for 3 days, at 37 °C in 95% air plus 5% CO_2. Stimulation was measured by the incorporation of tritiated thymidine (28.5 Ci/ mM, New England Nuclear, Massachusetts, USA) in the final 6 hr of culture. The stimulation index (SI) was calculated by dividing the mean counts per minute of the stimulated cells by those of the control cultures.

For PHA, and considering the results of Dr. M. Ulrich (IND, Caracas, Venezuela; personal communication) studying the normal Venezuelan population, the normal SI were considered to be >30.

For *C. albicans* and *P. brasiliensis* SI values greater than 2 standard deviations above the mean response of normal controls were considered as positive.

B-cell mediated immune response

Serum immunoglobulins

IgG, A and M were measured in the serum using the radial immunodiffusion method of Mancini *et al.* (17). The normal values were based upon those established by Merino and Brand in healthy Venezuelan individuals (20b).

Serum IgE concentrations were measured by the commercial RIST Phadebas test (Pharmacia, Uppsala, Sweden) and expressed as IU/ml. Considering the previous studies of Dessaint *et al.* (6) IgE concentrations greater than 570 IU/ml were considered significantly elevated.

Specific IgE antibodies against P. brasiliensis

These antibodies were measured in 7 of the mycologically active patients (Group 1) and in 7 of the inactive individuals (Group 2) by the radio-immunoadsorption technique previously described (33). The immunoadsorbent was prepared using a culture filtrate of strain CMM 4949.

Antibodies of other classes

To measure serum antibodies other than IgE, the specific double immunodiffusion technique of Yarzábal *et al.* (32), was used. Electrosyneresis as modified by Conti-Díaz *et al.* (3) and a microvariant of the enzyme-linked immunosorbent assay (ELISA) developed by Engvall and Perlman (7), were also employed.

The antigen used in ELISA was the filtered and dialysed medium of a 4 week old culture of *P. brasiliensis* strain IVIC* Pb 73, diluted in PBS 7.2 + 0.02% NaN_3 to a concentration of 50 ug/ml. The test was performed in microtitre plates (Cooke Lab. Products, Dynatech Labs., 1-220-24A, USA). The wells were sensitized using 200 ul of antigen solution for 48 hr at 4 °C. After washing 3 times with 250 ul of PBS + 0.05% Tween 20, the wells were filled with 20° ul of the test sera diluted in PBS + Tween, then incubated at 24–25 °C for 4 hr. After further washing, 200 ul of sheep anti-human Ig labelled with peroxidase (Institut Pasteur, Paris, France) were added for 18 hr at 4 °C. The wells were washed again, then 200 ul of the substrate (O-dianisidine + H_2O_2) added. After 60 min. incubation at 24–25 °C, the reaction was stopped by the addition of 5 ul of N HCl per well. The OD at 405 nm was then measured in a spectrophotometer with microcell attachments. The titre was taken as the greatest dilution that produced an OD greater than the mean + 2 SD of normal seral.

Statistical analysis

For statistical evaluation of the results, the non parametric Mann and Whitney U test was employed to compare the different groups.

Results

T-cell mediated immunity

The results of DNCB sensitization, intradermal skin tests and lymphocyte stimulation are presented in Tables 1 and 2. In Group 1 the 3 active patients with multifocal disseminated infection showed no reaction to any cutaneous test. Only 1 of the 10 members of this group responded to all the stimuli. The remainder showed variable reactivity.

In contrast, all the patients with mycologically inactive Pcm (Group 2) responded to 2 or more cutaneous tests; 2 reacted to 4, and 6 to 3 tests. Comparison between the groups (Table 1) shows that the percentage of positive tests in the inactive group is greater than in the active patients, the former being similar to the normal controls, with the exception of their positivity to soluble *P. brasiliensis* antigen.

Concerning lymphocyte stimulation, a depression was evident in the active group (Table 2). Thus, the lymphocytes of 4 of the 10 active patients had PHA SI less than 30; the non-viable particulate *P. brasiliensis* antigen provoked SI less than 3.8 (the

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Table 1. Proportion of cutaneous tests positive in 20 paracoccidioidomycosis patients and 8 controls.

Stimulant and dose	Patients Group 1		Group 2		Healthy controls Group 3	
	N° +	+ %	N° +	+ %	N° +	+ %
DNCB (25/50/100 μg)	4	40.0	6	60.0	ND	ND
PPD (2TU)	3	30.0	8	80.0	6	75.0
C. albicans (100 μg)	5	50.0	9	90.0	5	62.5
P. brasiliensis (40 μg)	4	40.0	7	70.0	2*	28.6

*n : 7
DNCB : Dinitrochlorobenzene
PPD : Purified protein derivative
ND : Not done

Table 2. Lymphocyte stimulation indices (range and median values) in 20 paracoccidioidomycosis patients and 8 controls.

Mitogens	Patients Group 1	Group 2	Healthy controls Group 3	Significance (p values) 1 vs 2	1 vs 3	2 vs 3
PHA	3.0–60.0 (37.50)	30.0–166.0 (70.00)	54.0–112.0 (86.25)	<0.01	<0.01	NS
C. albicans	1.0– 7.0 (1.80)	1.8– 13.7 (3.5)	1.0– 5.4 (4.20)	<0.02	NS	NS
P. brasiliensis	1.0–27.5 (3.10)	5.4– 43.0 (14.05)	1.0– 3.3 (2.00)	<0.01	NS	<0.001

* : Mann Whitney U test
PHA : Phytohaemagglutinin
NS : $\geqslant$0.05

Table 3. Leukocytes, lymphocytes and E-rosette forming cells in 20 paracoccidioidomycosis patients and 8 healthy controls (range and median values).

Cells	Patients Group 1	Group 2	Healthy controls Group 3	Significance (p values) 1 vs 2	1 vs 3	2 vs 3
Leukocytes/mm^3	6 050–13 200 (7 875)	4 900–12 900 (8 950)	4 200–8 000 (6 540)	NS	NS	0.02
Lymphocytes/mm^3	1 240– 4 356 (2 446)	2 058– 3 627 (2 841)	1 812–3 760 (2 304)	NS	NS	NS
E Rosettes/mm^3	657– 2 273 (1 186)	1 306– 2 456 (1 566)	1 093–2 303 (1 294)	NS	NS	<0.02

* : Mann Whitney U test
NS : >0.05

mean + 2 SD of normals) in 5 of the 10 group 1 patients, and none of this group had a greater SI than 7.52 (mean + 2 SD of normals) against soluble *C. albicans* antigen. In contrast, group 2 patients all had SI for PHA > 30, > 3.8 for *P. brasiliensis* and 3 of the 10 were >7.5 for *C. albicans*. Statistical analysis revealed that the PHA response was significantly depressed in group 1 compared to group 2 and controls ($p < 0.01$). Also, the difference in response to *C. albicans* and *P. brasiliensis* antigens was statistically significant between groups 1 and 2 ($p < 0.02$ and 0.01, respectively), the former responding as the non-sensitized normal group. In Table 3 are presented the lymphocyte and leukocyte counts, and the E rosette-forming cells in the 3 groups. In the treated patients there was a slight

augmentation in leukocyte number, as in the percentage of T lymphocytes, but differences between untreated patients and normal controls were not statistically significant.

B-cell mediated immunity

Quantitation of serum immunoglobulins

As can be seen in Table 4, the serum IgA and IgM concentrations showed no statistically significant differences in groups 1 and 2. The IgG levels were, however, generally higher in group 1. IgE levels were significantly elevated in groups 1 and 2, although not in all cases. Thus 3 of the 10 patients in group 1 and 5/10 in group 2 had levels lower than 570 IU/ml. The differences in IgE levels between the patients and healthy controls from the endemic area are highly statistically significant ($p < 0.01$).

Antibodies against P. brasiliensis

All serological tests (immunodiffusion, electrosyneresis and micro-ELISA) were positive in group 1 (Table 5). Seventy per cent of these patients had titres in ELISA of ≥1:1 600 ($\log_2 = 10.64$). The number of precipitation bands in immunodiffusion and electrosyneresis was generally high, the averages being 2.25 and 6.7 respectively. The presence of antibodies against antigen E_2 of *P. brasiliensis* was confirmed in all cases.

Patients of group 2 showed fewer immunoprecipitation bands and significantly lower ELISA titres than group 1 (Table 5). No control serum was positive in these tests.

IgE antibodies against P. brasiliensis antigens

The mean specific IgE antibody concentrations (IU/ml) were 16.24 (range 1.5 to 78.0) in patients with active disease, and 4.76 (range 2.4 to 6.6) in individuals with inactive infection (Table 5). These values represent only 0.6% of the mean total IgE concentrations in group 1, and 0.4% in group 2.

Table 4. Concentration of serum immunoglobulins (range and median values).

Immunoglobulins	Patients		Healthy controls	Significance (p values)*		
	Group 1	Group 2	Group 3	1 vs 2	1 vs 3	2 vs 3
IgG (mg %)	1 020–2 980 (1 910)	700–1 400 (1 130)	– (1 475)	<0.05	ND	ND
IgA (mg %)	125– 475 (280)	120– 400 (267)	– (324)	NS	ND	ND
IgM (mg %)	110– 240 (130)	115– 190 (154)	– (146)**	NS	ND	ND
IgE (IU/ml)	103–7 600 (1 471)	45–4 199 (631)	11–453 (136)	NS	<0.01	<0.05

* : Mann Whitney U test
** : Data from Merino and Brand (20b)
NS : >0.05
ND: Not done

Table 5. Anti-*P. brasiliensis* antibodies detected by immunodiffusion (ID), electrosyneresis (ES), microelisa and radioimmunoadsorption (RIA) (range and median values).

Sera	ID (n°-bands)	ES (n°-bands)	MICROELISA ($\log_2$ titres)	RIA (IU IgE/ml)
Group 1	1.0–7.0 (1.50)	4.0–9.0 (6.50)	8.64–13.64 (11.14)	1.5–78.0 (5.30)
Group 2	1.0–3.0 (1.00)	0.0–9.0 (3.00)	7.64–11.64 (8.64)	2.4– 6.6 (4.40)
Group 3	0.0	0.0	<7.64	0.0

Discussion

The results obtained here confirm previous reports that active Pcm produces diverse grades of depression of T-cell function, that are both specific and non-specific and reversed by treatment with sulphonamides and/or amphotericin B. Thus, T-cell depression has been previously described in Brazilian and Colombian patients, and posttherapeutic recovery of activity has also been reported (19, 20a, 21, 23, 28).

The various published studies have used a variety of techniques and materials, and comparisons should be made with caution. It is, however, relevant to consider these previous studies further.

Concerning DNCB sensitization, it has been reported that this is negative in 64 to 73% of Pcm patients (19, 20a, 21, 23). We found 60% non-reactivity in active, and 40% in inactive cases. The suppression appears to be, therefore, at least partially reversible.

PPD responses were positive in 26% of the patients studied by Musatti *et al.* (23), and 19% of those of Restrepo *et al.* (28) at the time of diagnosis. After 6 months of treatment this rose to 56% in the latter group. We observed 10% positive in the active group and 60% in those treated. These results are, therefore, in agreement with those of Restrepo *et al.* (28).

A more variability has been reported in the response to *C. albicans,* ranging from 30 to 61% positive in Brazilian patients. Restrepo *et al.* (28) observed an increase from 56% to 81%, after treatment. We confirmed these later results as 56% of our active patients were positive and this reactivity rose to 90%. Our group 2 was, however, not necessarily directly comparable to group 1, since our patients were not studied longitudinally.

The cutaneous response to *P. brasiliensis* antigens was first studied by Fonseca and Leao (8). It was soon reported that the patients with severe forms of the mycosis did not develop delayed type skin reactions, but exhibited this ability after successful treatment. Restrepo *et al.* (28), using a partially purified and standardized antigen, found reactivity read at 24 hours increasing from 12% to 36% after 6 months or more of treatment. The differences in the percentage of positive reactions between our study and that of Restrepo *et al.* (28) may be due to the small sample number (10 patients in each group in our study) or to different severities or treatment times.

Considering in general terms our results of cutaneous testing, the importance of the severity of the disease is evident. Thus in the active group, the 3 patients with multifocal dissemination were negative to the 4 tests, while the 3 patients with pulmonary localization responded to 3 of the 4 tests on average.

The polyclonal stimulation of lymphocytes by PHA has been examined in Pcm since the beginning of the 1970's. Mendes *et al.* (19, 20a) and Musatti *et al.* (23) examining 3 H-thymidine incorporation found diminished responses in the progressive form of the disease. Mok & Greer (21) found, however, no differences between symptomatic patients and healthy individuals. Restrepo *et al.* (28) using acridine orange staining, examined PHA responses and found significant differences between patients (treated and non-treated) and healthly controls. We found depression in the active patients, but normal responsiveness in others that had been successfully treated.

Antigen-specific *in vitro* stimulation has also been studied using homologous and heterologous antigens. We examined soluble *C. albicans* extracts and soluble or particulate antigens of *P. brasiliensis.* Responses to *C. albicans* appeared to be diminished in the active patients, but augmented in the inactive group, when compared to healthy controls. This elevation in the inactive group has been previously reported by Musatti *et al.* (23) and may be due to cross reaction with *C. albicans* antigens shared by *P. brasiliensis.*

Concerning responsiveness to *P. brasiliensis* itself, the results depended to a considerable extend upon the form of the antigen. Culture medium filtrate gave low stimulation indices, as has also been reported by Mendes *et al.* (19, 20a), Mok & Greer (21) and Musatti *et al.* (23). Viable yeast-forms were inhibitory, but killed whole organisms were very active. Using the latter preparation, the response of the patients was readily distinguishable from the unresponsiveness of the healthy controls, and differences were discernable between active and inactive patients. Thus, like Restrepo *et al.* (28), we can suggest an apparent recovery of the capacity to respond to specific stimulation by lymphocytes of Pcm patients after treatment, although a longitudinal study is warranted to confirm our observation.

We did not study the inhibitory effect of viable yeasts of *P. brasiliensis,* although it can be suggested that this was due to competition for 3H-thymidine or nutrients, or via the liberation of soluble factors which may exert, or stimulate, toxic or suppressor cell effects. This aspect of our results requires further investigation.

In contrast to other authors (23, 28), we did not find significant differences in the number of peripheral T lymphocytes between the 3 groups examined.

In our studies of B-cell function, we confirmed the previously described augmentation in serum IgG concentrations, and the existence of high levels of specific antibodies against the fungus, in patients with active disease. In contrast, IgA and IgM levels were within the normal range in both active and inactive groups. Other authors have described increased levels of IgG in Pcm, being maintained for long periods and correlating with complement fixing antibodies (4, 28). We demonstrated a not unexpected concordance between the number of bands in immunoprecipitation and titres in ELISA, these being significantly greater in the active patients, and possibly associated with depressed cell mediated immunity. Correa and Giraldo (4) and Restrepo *et al.* (28) also observed unchanged serum IgA levels; however, considering that Pcm often involves the respiratory apparatus, local IgA quantitation would be relevant in further studies.

The normal IgM levels may be related to the chronic form of the disease, and the long period between infection and examination and eventual diagnosis. It should be mentioned, however, that specific IgM antibodies may have been present in our patients, as was recently demonstrated by Mota and Franco (22) in 68% of their Pcm sera.

One of the most interesting of our observations was the high serum IgE levels. Such elevation has been previously reported by us in the sera from active patients (33), and similar results have also been recently found in coccidioidomycosis (5), chronic dematohytosis (10) and candidiasis (18). The highest IgE levels were found in active Pcm patients, in whom cellular immunity was generally depressed, suggesting a relation between T-cell dysfunction and hyperproduction of IgE. It should be noted, however, that the influence of intestinal helmintic infection in these elevated levels can not be strictly evaluated, as faecal examination was not performed: despite this, the considerably augmented levels in the Pcm patients are of interest, as the normal control values were determined in individuals of the same geographic and racial origin, socioeconomic status, living in the endemic area. The actual proportion of the serum IgE that was specific antibody against *P. brasiliensis* was very low, representing only 0.6% of the total IgE.

This hyperimmunoglobulinemia E may be due either to the existence of allergic reactions, activation of B cells, or a failure in T-cell regulation mechanisms. Clinical symptoms of allergy and IgE antibody levels against other antigens should be further investigated to clarify eventual involvement of atopy. The presence of non-specific B-cell stimulation can be neither affirmed nor rejected from our results. However, the high levels in active patients, the lower levels in treated individuals, and the association with depressed cell-mediated immunity suggest that T-cell dysfunction might contribute to the excessive IgE production. Studies in progress in our laboratory suggest that circulating immune complexes may be involved in the apparent T-cell defect.

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